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Transmitted herewith for filing under 37 CFR §1.53(c) is the PROVISIONAL APPLICATION for patent of

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TITLE OF THE INVENTION (280 characters max)

USE OF GENETIC POLYMORPHISMS THAT ASSOCIATE WITH EFFICACY OF TREATMENT OF INFLAMMATORY DISEASE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification (Including Any Claims and Abstract) - 30 pages
☒ Drawings - 1 sheets
☒ Other (specify): Application Data Sheet

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Respectfully submitted,

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Date: October 6, 2003

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USE OF GENETIC POLYMORPHISMS THAT ASSOCIATE WITH EFFICACY OF TREATMENT OF INFLAMMATORY DISEASE

FIELD OF THE INVENTION

[0001] This invention relates generally to the analytical testing of tissue samples *in vitro*, and more particularly to the analysis of genetic polymorphisms as biomarkers for the efficacy of treatments of inflammatory disease.

BACKGROUND OF THE INVENTION

[0002] Atopic dermatitis (AD), also known as eczema, is an inflammatory skin disease typified by itchy inflamed skin that presents during infancy and childhood. The disease is one of the most common dermatological disorders in Western countries, affecting as many as 20% of children under the age of five. Cookson WO *et al., Nature Genetics* 27: 372-373 (2001).

[0003] Pimecrolimus (Elidel®) is an ascomycin macrolactam derivative that was specifically developed for the treatment of inflammatory skin diseases. Tacrolimus (FK506; Protopic® or Prograf®) is a member of the same class of compounds and is also used to treat inflammatory skin diseases. Both pimecrolimus and tacrolimus interfere with the inflammatory process by binding with high affinity to macrophilin-12 and inhibiting calcineurin, which blocks transcription of Th1- and Th2-type cytokines in T lymphocytes. While the two compounds are structurally similar and have comparable mechanisms of action, pimecrolimus has a higher affinity for skin than does tacrolimus.

[0004] It has long been recognized that there is a genetic basis to atopic diseases. Several linkage analyses have been done to define susceptibility regions that contribute to atopic dermatitis, as reviewed in Maclean JA & Eidelman FJ, *Arch Dermatol* 137: 1474-1476 (2001). At least five chromosomal regions (3q21, 5q31-33, 11q13, 13q12-14 and 14q11.2) are thought to contain atopic dermatitis susceptibility loci. Current thinking is that atopic mechanisms are primarily responsible for the pathogenesis of the atopic dermatitis. However, other genes involved with dermal inflammation may also contribute to the condition. Cookson WO *et al., Nature Genetics* 27: 372-373 (2001).

[0005] The response rate among patients taking topical macrolactams (pimecrolimus or tacrolimus) is currently less than 50%. It is not known why some patients do not respond to these medications. Thus, there remains a need in the art for the improving the efficacy of topical macrolactams, such as by targeting appropriate formulations of the medications to those inflammatory disease patients who are genetically disposed to respond and benefit from the treatment.

SUMMARY OF THE INVENTION

[0006] The invention provides a method for treating atopic dermatitis, based upon a determination of the patient's *TNF* alleles. In one embodiment, patients are tested for the presence of a (-1031) *TNF* polymorphism to determine what therapy is appropriate. Patients having the TT alleles at that locus are treated with pimecrolimus cream or ointment, while patients having the CC alleles or the CT alleles are treated with a higher dose of pimecrolimus cream, with pimecrolimus cream plus another anti-inflammatory drug, or with an anti-inflammatory drug without pimecrolimus cream.

[0007] The invention also provides a general method for treating psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or any disorder for which pimecrolimus (oral, topical or other) is indicated, based upon a determination of the patient's *TNF* alleles. In one embodiment, patients are tested for the presence of a (-1031) *TNF* polymorphism to determine what therapy is appropriate. Patients with TT alleles at that locus are treated with pimecrolimus, while patients with CC alleles and CT alleles are given a higher dose of pimecrolimus, pimecrolimus plus another anti-inflammatory drug or an anti-inflammatory drug without pimecrolimus.

[0008] The invention further provides a method for treating psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or any disorder for which pimecrolimus (oral, topical or other) is indicated, based upon a determination of the patient's *LTA* alleles. In one embodiment, patients are tested for the presence of an ASN60THR *LTA* locus polymorphism to determine what therapy is appropriate. Patients with AA or AC alleles at that locus are treated with pimecrolimus, while patients with CC alleles are given a higher dose of pimecrolimus, pimecrolimus plus another anti-inflammatory drug or an anti-inflammatory drug without pimecrolimus.

[0009] The invention also provides a method for treating psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or any disorder for which tacrolimus (oral, topical or other) is indicated, based upon a determination of the patient's *CCR2* alleles. In one embodiment, patients are tested for the presence of a VAL64ILE *CCR2* locus polymorphism to determine what therapy is appropriate. Patients with GG alleles at that locus are treated with tacrolimus, while patients with AG alleles are given a higher dose of tacrolimus, tacrolimus plus another anti-inflammatory drug or an anti-inflammatory drug without tacrolimus.

[0010] The invention provides a method for treating atopic dermatitis or any disorder for which pimecrolimus (oral, topical or other) is indicated, where the level of TNF-α protein or mRNA present in the patient is measured prior to determining what therapy is appropriate. In one embodiment, when TNF-α protein or mRNA levels in the bodily fluids or non-inflamed tissue samples of the patient are low or normal, then the patient is treated with pimecrolimus. In another embodiment, when TNF-α protein or mRNA levels are elevated, then the patient is treated with pimecrolimus in combination with another anti-inflammatory drug or with an alternative therapy.

[0011] The invention provides a method for choosing subjects for inclusion in a clinical trial of inflammatory skin disease or any other disorder for which pimecrolimus is indicated, where genetic polymorphisms at the (-1031) TNF locus, the ASN60THR *LTA* locus or the VAL64ILE *CCR2* locus of the candidate subjects are interrogated prior to inclusion in the clinical trial.

[0012] The invention provides a kit for use in determining treatment strategy for a patient with inflammatory skin disease or any other disorder for which pimecrolimus is indicated. In one embodiment, the kit contains reagents for determining the levels of TNF-α protein or mRNA in a sample obtained from a subject to be treated. In another embodiment, the kit contains reagents for determining the genotype of the subject in the *TNF, LTA* or *CCR2* genes. The kit also contains a written product describing the use of the biomarker in determining a treatment strategy for the condition.

[0013] The invention thus provides a way of improving the efficacy of topical macrolactams, particularly pimecrolimus, by identifying biomarkers of efficacy in the subjects to be treated and then targeting appropriate macrolactam formulations to those who are best able to respond and benefit from the treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 is a diagrammatic representation of the major histocompatability complex (MHC) on chromosome (Ch) 6 showing the placement of LTA (gene coding for lymphotoxin alpha) and TNF (gene coding for tumor necrosis factor alpha; TNF-α) within the MHC. Shown in greater detail below is the region of 6p21.3 that contains the TNF gene cluster. * denotes polymorphisms, which are roughly 2.5 kb apart, discussed herein. This figure is modified from Field M, *Q J Med* 94: 237-246 (2001).

DETAILED DESCRIPTION OF THE INVENTION

[0015] The efficacy of treatment of atopic dermatitis by pimecrolimus cream 1%, but not treatment by tacrolimus ointment 0.03%, is associated with polymorphic markers in the *TNF* cluster on 6p21.3. This discovery provides a way to use genetic polymorphisms that associate with the efficacy of pimecrolimus treatment in determining the most effective treatment.

[0016] The genetic locus was identified in a Phase IIIb trial that was designed as a head to head comparison of pimecrolimus cream 1% and tacrolimus ointment 0.03%. The six-week investigator-blinded trial of pediatric subjects with moderate atopic dermatitis explored the incidence of local application site reactions induced by the two topical macrolactams, as well as their efficacy, safety and cosmetic acceptability. Blood samples for pharmacogenetic analysis were collected from consenting participants in the clinical trial, to define a genetic basis for response to either treatment.

[0017] Among those genes selected for pharmacogenomic analysis were *LTA*, which codes for lymphotoxin alpha (LTα), and *TNF*, which codes for tumor necrosis factor alpha (TNF-α). The *LTA* and *TNF* genes are tandemly located in the gene-rich MHCIII region of 6p21.3 (see, FIG. 1). Both genes contain multiple polymorphisms, some of which have been well-characterized, as reviewed by Field M, *Q J Med* 94: 237-246 (2001). Both the *LTA* and the *TNF* gene products are cytokines involved in inflammatory processes. Both cytokines signal through TNF-α receptors, which are expressed in many different tissues, including the skin. Rulls SR & Sedgwick JD, *Am J Hum Genet* 65: 294-301 (1999). Furthermore, both *LTA* and *TNF* have been implicated in the pathogenesis of atopic disease. Polymorphisms in both these genes are associated with atopy, particularly asthma. Trabetti E *et al., J Med Genet* 36: 323-5 (1999);

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Izakovicaova Holla L *et al., Clin Exp Allergy* 9: 1418-23 (2001); Castro J *et al., J Investig Allergol Clin Immunol* 3:149-5 (2000); Li Kam Wa TC *et al., Clin Exp Allergy* 29: 1204-8 (1999); and Zhu S *et al. Am J Respir Crit Care Med* 161: 1655 – 9 (2000). TNF-α has been extensively studied for its role in dermal inflammation. Both dermal mast cells and keratinocytes produce TNF-α. While expression of TNF-α is constitutive in the skin, the level of expression can be further induced by a variety of stimuli. TNF-α levels are elevated in skin lesions of psoriasis, and several reports have suggested that drugs that modulate TNF-α function are effective in the treatment of psoriasis, as reviewed by LaDuca JR & Gaspari AA, *Dermatol Clin* 19: 617-635 (2001) and Mease PJ, *Ann Rheum Dis* 61: 298-304 (2002). Though psoriasis is distinct from atopic dermatitis, the expression of both diseases is influenced by genes that modulate dermal inflammation. Cookson WO *et al., Nature Genetics* 27: 372-373 (2001).

[0018] In the clinical trial and subsequent pharmacogenomics analysis, an association between pimecrolimus efficacy and the (-1031) *TNF* polymorphism was found in pediatric subjects (ages 2-17 years) with atopic dermatitis who were treated topically with the cream formulation of pimecrolimus. The polymorphism that associated with pimecrolimus efficacy is located –1031 from the transcription start site of the gene and changes the wild-type T to a C. The only subjects that experienced efficacy in this trial had a TT genotype at (-1031) *TNF*. No patient with a C allele (CC or CT) at that locus responded to pimecrolimus. This association did not hold for tacrolimus.

[0019] For a second SNP in the same chromosomal region, ASN60THR *LTA*, the data also suggested a trend toward significance.

[0020] These associations can reasonably be expected to hold for all age groups and for subjects with other inflammatory skin diseases. Moreover, the efficacy of pimecrolimus can reasonably be predicted when the compound is formulated as a tablet or aerosol. The method can be used for vertebrate subjects, particularly mammalian subjects and more particularly for human subjects.

[0021] Individuals carrying polymorphic alleles of interest (*i.e.*, in genes from the *TNF* cluster, such as *TNF* and *LTA*) may be detected at the DNA, the RNA, or the protein level using a variety of techniques that are well known in the art. Strategies for identification and detection are described in *e.g.* EP 730,663, EP 717,113, and PCT US97/02102. The methods of the invention

may involve the detection of pre-characterized polymorphisms, where the genotyping location and nature of polymorphic forms present at a site have already been determined (see, discussion above regarding *LTA* and *TNF* genes). The availability of this information allows sets of probes to be designed for specific identification of the known polymorphic forms. The identification of alleles containing single nucleotide polymorphisms may involve the amplification of DNA from target samples. This can be accomplished by *e.g.*, PCR. See generally *PCR Technology: Principles and Applications for DNA* Amplification, (ed. Erlich, Freeman Press, NY, N.Y., 1992); *PCR Protocols: A Guide to Methods and Applications* (eds. Innis, *et al.*, Academic Press, San Diego, Calif., 1990). The detection of polymorphisms in specific DNA sequences, can be accomplished by a variety of methods including, but not limited to, restriction-fragment-length-polymorphism detection based on allele-specific restriction-endonuclease cleavage (Kan & Dozy, *Lancet* II:910-912 (1978)), hybridization with allele-specific oligonucleotide probes (Wallace *et al, Nucl. Acids Res.* 6:3543-3557 (1978)), including immobilized oligonucleotides (Saiki e*t al.*, *Proc. Natl. Acad. Sci. USA*, 86:6230-6234 (1969)) or oligonucleotide arrays (Maskos & Southern, *Nucl. Acids Res.* 21:2269-2270 (1993)), allele-specific PCR (Newton et *al., Nucl. Acids Res.* 17:2503-2516 (1989)), mismatch-repair detection (MRD) (Faham & Cox, *Genome R*es. 5:474-482 (1995)), binding of MutS protein (Wagner *et al., Nucl. Acids Res.* 23:3944-3948 (1995), denaturing-gradient gel electrophoresis (DGGE) (Fisher & Lerman, *Proc. Natl. Acad. Sci. U.S.A.* 80:1579-1583 (1983)), single-strand-conformation-- polymorphism detection (Orita *et al., Genomics* 5:874-879 (1983)), RNAse cleavage at mismatched base-pairs (Myers *et al., Science* 230:1242 (1985)), chemical (Cotton *et al., Proc. Natl. Acad. Sci. U.S.A.*, 8Z:4397-4401 (1988)) or enzymatic (Youil *et al., Proc. Natl. Acad. Sci. U.S.A.* 92:87-91 (1995)) cleavage of heteroduplex DNA, methods based on allele specific primer extension (Syvanen et *al., Genomics* 8:684-692 (1990)), genetic bit analysis (GBA) (Nikiforov *et al., Nucl. Acids Res.* 22:4167-4175 (1994)), the oligonucleotide-ligation assay (OLA) (Landegren *et al., Science 241*:1077 (1988)), the allele-specific ligation chain reaction (LCR) (Barrany, *Proc. Natl. Acad. Sci. U.S.A.* 88:189-193 (1991)), gap-LCR (Abravaya *et al., Nucl. Acids Res.* 23:675-682 (1995)), radioactive and/or fluorescent DNA sequencing using standard procedures well known in the art, and peptide nucleic acid (PNA) assays (Orum *et al., Nucl. Acids Res.* 21:5332-5356 (1993); Thiede e*t al., Nucl. Acids Res.* 24:983-984 (1996)). Additional guidance is provided by Sambrook J *et al.*,

Molecular Cloning: A Laboratory Manual, Third Edition (Cold Spring Harbor Press, Cold Spring Harbor, 2000).

[0022] Levels of TNF-α mRNA or protein can be determined by methods known to those of skill in the art. See, U.S. Pat. Nos. 6,566,501; 6,541,620; and 6,537,540. A discussion of the levels of the level of tumor necrosis factor in TNF-associated disorders is provided in U.S. Pat. No. 6,537,540 and references cited therein (all of which are incorporated by reference.).

[0023] As used herein, the level of TNF-α mRNA in the sample is determined to be "high" when the ratio of TNF-α mRNA to β-actin expression (See, Actor JK *et al., Comb Chem High Throughput Screen* 3(4):343-51 (August 2000)) or GAPDH expression (See, Anderson GD *et al., J Clin Invest.* 97(11):2672-9 (June 1, 1996)) in the sample is about twice as high or higher than the ratio of TNF-α mRNA to β-actin expression, as compared with a sample from an individual (or preferably a population of individuals) who does not have an inflammatory skin condition. As used herein, the level of TNF-a mRNA in the sample is determined to be "low or normal" when the ratio of TNF-α mRNA to β-actin expression in the sample is about equal to or less than the ratio of TNF-α mRNA to β-actin expression, as compared with a sample from an individual (or preferably, a population of individuals) who does not have an inflammatory skin condition. For guidance as to which TNF-α mRNA and protein levels tissue are generally expected to be "low", "normal" or "high", see, Van Deventer SJH, *Gut* 40: 443-8 (1997); McAlindon ME & Mahida YR, *Aliment Pharmacol Ther* 10(suppl 2): 72-4 (1996); Reimund J-M *et al., J Clin Immunol* 16: 144-50 (1996); Reinecker H-C *et al., Clin Exp Immunol* 94: 174-81 (1993); Murch SH *et al., Gut.* 34: 1705-9 (1993) and Grom AA *et al., Arthritis Rheum* 39: 1703-1710 (1996). Alternatively, one of skill in the art can define an average or expected level of TNF-α mRNA expression in a population, *e.g.*, by performing the assay in ten or more control subjects (such as by methods shown in the EXAMPLE) and obtaining the average and standard deviation.

[0024] Samples for the determination of genetic polymorphisms, *TNF* mRNA levels or TNF protein levels can be collected from subjects by any appropriate method know to those of skill in the medical art. Samples can be fluid samples, such as from blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen or other bodily fluid. Alternatively, samples can be tissue samples, such as from biopsy samples, homogenate, lysate or extract prepared from a whole organism or a subset of its

tissues, cells or component parts, or a fraction or portion thereof. A sample can also be a medium, such as a nutrient broth or gel in which an organism has been propagated, which contains cellular components, such as proteins or nucleic acid molecules.

[0025] Guidance for the use of pimecrolimus cream 1% is provided by Novartis Pharmaceuticals Corp., Elidel® Prescribing Information, T2003-01, 89014902, 492573/1 US (Rev. April 2003). Additional guidance for the use of pimecrolimus is provided by Nghiem P *et al., J Am Acad Dermatol* 46: 228-241 (2002) and references cited therein (all of which are incorporated by reference) and in U.S. Pat. Nos. 5,912,238; 6,352,998 and 6,423,722.

[0026] Guidance for the use of tacrolimus ointment (0.03% or 0.1%) is provided by Fujisawa Healthcare Inc., Protopic® Prescribing Information, 45670 (December 2000). Guidance for the use of Prograf® (oral capsule or injectable) is provided by Fujisawa Healthcare Inc., Prograf® Prescribing Information, ZL40306 and Fujisawa Healthcare Inc., Prograf® Product Monograph. Additional guidance for the use of tacrolimus is provided by Nghiem P *et al., J Am Acad Dermatol* 46: 228-241 (2002) and references cited therein (all of which are incorporated by reference).

[0027] As used herein, a "high dose of pimecrolimus" is a dose substantially higher than the recommended dose of pimecrolimus. For example, for adults and children over two years of age, a thin layer of pimecrolimus cream 1% is applied to the affected area twice times a day, then rubbed in gently. A dose substantially higher than the recommended dose may be the application four times a day. Alternatively, a dose substantially higher than the recommended dose may be the application twice a day of a pimecrolimus formulation with a concentration of 2% or higher, if such be made available. Likewise, a "high dose of tacrolimus" is a dose substantially higher than the recommended dose of tacrolimus.

[0028] The use of other immunosuppressants, such as hydrocortisone, cyclosporine or sirolimus (rapamycin), in dermatology generally and in particular for atopic dermatitis, is well-known to those of skill in the medical arts. See, Marsland AM & Griffiths CE, *Eur J Dermatol.* 2(6):618-22 (November-December 2002) and Nghiem P *et al., J Am Acad Dermatol* 46: 228-241 (2002).

[0029] The diagnosis of atopic dermatitis is well-known to those of skill in the medical arts. See, Cookson WO *et al., Nature Genetics* 27: 372-373 (2001); Nghiem P *et al., J Am Acad*

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Dermatol 46: 228-241 (2002) and references cited therein (all of which are incorporated by reference).

[0030] The kits of the invention may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit to predict whether a patient will respond effectively to macrolactam treatment, especially pimecrolimus treatment. In several embodiments, the use of the reagents can be according to the methods of the invention.

EXAMPLE

PHARMACOGENETIC ANALYSIS OF PATIENT RESPONSE TO THERAPY IN CLINICAL TRIAL: POTENTIAL ASSOCIATION OF PIMECROLIMUS EFFICACY WITH POLYMORPHISMS IN THE TNF GENE CLUSTER

[0031] *Genotyping selected candidate genes*. Forty-six patients, corresponding to 24 patients who received tacrolimus and 22 patients who received pimecrolimus, were analyzed for possible associations between genetic markers and treatment efficacy.

[0032] Thirty-one unique polymorphic sites in a total of 22 genes were analyzed. Table 1 lists the genotype frequencies for each SNP examined in the trial population that consented to participation in the pharmacogenetic analysis. Each SNP is identified by three pieces of information: (1) REF_ACC, or the Genbank accession number where genomic sequence for the gene can be found, (2) Position, which refers to the position of the nucleotide within the REF_ACC that was interrogated, and (3) TWT SNP#, which refers to the number assigned by Third Wave Technologies to the assay that was designed to assess the genotype at the position defined by items 1 and 2. Location refers to the site within the gene that harbors the polymorphism, if known.

Table 1
List of polymorphisms examined in the pharmacogenomic analysis

Gene Symbol	Gene Name	TWT SNP #	REF ACC	Allele 1 Freq.	Allele 2 Freq.	Position	Location
ACE	Angiotensin I converting enzyme	103199	AF1178569	0.50(T)	0.50(C)	10514	
ACE	Angiotensin I converting enzyme	103200	AF1178569	0.56(A)	0.44(G)	14521	
CCR2	chemokine (C-C motif) receptor 2	103240	U80924	1.0(A)	0.00(G)	4201	3' UTR
CCR2	chemokine (C-C motif) receptor 2	47987	U95626	0.77(G)	0.23(A)	46295	Exon 1
CCR5+	chemokine (C-C motif) receptor 5	128004	U95626	1.00(T)	0.00(A)	61785	Exon 2
CCR5	chemokine (C-C motif) receptor 5	128005	U95626	0.60(A)	0.40(G)	62035	promoter
CTLA4	cytotoxic T-lymphocyte-associated protein 4	128016	M74363	0.56(A)	0.44(G)	1241	Exon 1
*ICAM1**	intercellular adhesion molecule 1	128063	X59287	0.98(A)	0.02(T)	120	Exon 2
*ICAM1**	intercellular adhesion molecule 1	128062	X59288	0.98(G)	0.02(T)	659	Exon 4
IFNG	Interferon gamma	229376	J00219	0.65(A)	0.35(G)	5644	promoter
IFNGR1+	Interferon gamma receptor 1	229419	U19241	1.00(G)	0.00(A)	4020	Exon 1
IFNGR2	Interferon gamma receptor 2	252011	AP000113	0.84(A)	0.16(G)	42786	Exon 2
IL10	Interleukin 10	229400	X78437	0.67(A)	0.33(G)	8210	5'UTR
IL3	Interleukin 3	251975	AF365976	0.61(C)	0.39(T)	1990	Exon 1
IL3+	Interleukin 3	229368	AF365976	1.00(T)	0.00(C)	3622	
IL4R	Interleukin 4 receptor	229406	AF421857	0.52(C)	0.48(T)	13715	Intron 5
*IL5**	Interleukin 5	229372	AF353265	0.98(G)	.02(A)	2718	
IL8	Interleukin 8	229405	AF385628	0.55(C)	0.45(T)	4501	Intron 2
ITGB2+	Integrin, beta 2 (LFA1)	128081	X64075	1.00(G)	0.00(A)	64	Exon 5
LTA	Lymphotoxin alpha	229383	M55913	0.59(C)	0.41(A)	800	Exon 3
*LTB**	Lymphotoxin beta	128095	L11016	0.91(C)	0.09(A)	5452	

Table 1 (Continued)
List of polymorphisms examined in the pharmacogenomic analysis

Gene Symbol	Gene Name	TWT SNP #	REF_ACC	Allele 1 Freq.	Allele 2 Freq.	Position	Location
MICA	MHC class I polypeptide-related sequence A	229390	AF336081	0.98(A)	0.02(G)	106	Exon 2
MICA	MHC class I polypeptide-related sequence A	251974	AF336081	0.78(A)	0.22(G)	1612	Exon 5
MICA	MHC class I polypeptide-related sequence A	229395	AF336081	0.76(G)	0.24(A)	658	Exon 3
NFATC2	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	252013	AL035682	0.49(A)	0.51(G)	58458	
PLGC1	Phospholipase C γ-1	229389	AL022394	0.69(C)	0.31(T)	64001	
TGFB1	Transforming growth factor β-1	128146	X05839	0.73(C)	0.27(T)	629	promoter
TNF	Tumor necrosis factor α	128152	M16441	0.79(T)	0.21(C)	3064	promoter
TNF	Tumor necrosis factor α	128150	M16441	0.85(C)	0.15(T)	3238	promoter
TNF	Tumor necrosis factor α	128148	M16441	0.80(G)	0.20(A)	3787	promoter
TNFSF6	tumor necrosis factor receptor superfamily, member 6	128153	X81335	0.58(G)	0.42(A)	1110	promoter

+ This SNP is not polymorphic in this patient population.

* This population is not in Hardy Weinberg equilibrium for this SNP.

[0033] The *FKBP1A* gene, which codes for FK506 Binding Protein 1A (macrophilin-12), the target of pimecrolimus and tacrolimus (FK506), was not genotyped, because the gene was found to contain no SNPs. No polymorphic sites in *FKBP1A* have been reported in public databases. We sequenced the *FKBP1A* gene to look for unknown SNPs, but found none.

[0034] To interrogate the genotype at each locus of interest, probe sets flanking the SNP were designed using publicly available databases such as OMIM, the SNP Consortium, LocusLink and dbSNP. The probe sets were synthesized by Third Wave Technologies, Inc. (TWT, Madison WI). Genotyping was performed with 60 ng of genomic DNA using the Invader® assay developed by Third Wave Technologies according to the manufacturer's

instructions. Lyamichev V et al., *Nat Biotechnol* 17: 292-6 (1999) and Ryan D, *Mol Diagn* 4: 135-44 (1999).

[0035] *Genotyping of ASN60THR LTA*. To confirm the results of the Third Wave Technologies genotyping assay for ASN60THR *LTA* (TWT #229383), a nested polymerase chain reaction (PCR) approach was used. All oligonucleotides were purchased from Research Genetics (Huntsville, AL). First, a 481 bp fragment was amplified using primers with the following sequences: forward (5'-acaccacctgaacgtctcttc-3'; SEQ ID NO:1) reverse (5'-tctcaatccctgaggaagtgg-3'; SEQ ID NO:2). See Genbank accession number M55913 for the complete *LTA* sequence. The amplicon was purified using Microcon 100 columns (Amlicon, Beverly, MA) and used as a template to amplify a 163 bp fragment using primers with the following sequences: forward (5'-tcagccaaaccttgagccctagag–3'; SEQ ID NO:3) and reverse (5'-atgtttaccaatgaggtgagcagcaggtttgcgg-3'; SEQ ID NO:4). Both PCR reactions were carried out in a Perkin Elmer 9700 thermocycler using 80 ng of genomic DNA, 60 ng of each primer, 1.0 Unit (U) of AmpliTaq polymerase (Perkin Elmer), and 200 μM dNTP (Pharmacia, Piscataway, N.J.) in a buffer consisting of 50 mM KCl, 10mM TRIS-HCl (pH 8.3), 1.5 mM MgCl and 0.01% gelatin. The PCR cycles for both amplifications consisted of the following: 94°C, 5 min; 94°C, 30 sec, 57°C, 30 sec, and 72°C, 30 sec for 35 cycles; 72°C, 10 minutes, then 4°C. The 163 bp amplicon was digested with Fau I (New England Biolabs, Beverly MA), and electrophoresed on a 4% agarose gel. A G allele produced an uncut 163 bp fragment, while an A produced 35 and 128 bp fragments. Both the 128 and 163 bp fragments were seen in heterozygote individuals. All but two of the genotypes determined by Third Wave Technologies technology could be confirmed by restriction digest.

[0036] *Genotyping of (-1031) TNF*. To confirm the results of the genotyping assay for *TNF* -1031 (TWT #128152), a 1113 bp fragment of the *TNF* promoter was PCR amplified and sequenced for each sample. PCR primers had the following sequences: forward (5'-TGGGAGTGAGAACTTCCCAG-3'; SEQ ID NO:5) and reverse (5'-TGAGCTCATCTGGAGGAAGC-3'; SEQ ID NO:6). See Genbank accession number M16441 for the complete sequence of human *TNF*. PCR reactions were carried out using the same conditions described above. PCR products were purified through Microcon 100 (Amlicon, Beverly, MA) columns.

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[0037] Following purification of the amplicon, 5 ng per 100 bp were used for sequencing with the ABI Prism Dye Terminator Cycle Sequencing kit as recommended (Perkin Elmer, Foster City, CA). Sequencing reactions contained either forward (5'-TGGGAGTGAGAACTTCCCAG-3'; SEQ ID NO:7) or reverse (5'-CTTAAACGTCCCCTGTATTC-3'; SEQ ID NO:8) primer and were run as follows: 96°C, 5 min; 96°C, 10 sec, 50°C, 5 sec, and 60°C, 4 min for 25 cycles; then 4°C. Sequencing reactions were purified with Centrisep spin columns (Princeton Separation, Adelphia, NJ) and run on the ABI 373A sequencer. DNA sequences were analyzed using ABI Prism Sequence Analysis V3.3 software.

[0038] *Statistical analysis*. Investigator global assessment (IGA) scores were used as the primary marker of efficacy in the pharmacogenetic studies. IGA scores at the end of the Investigator Blinded phase of the trial (Day 43) were used as the main time-point for comparison. Last observation carried forward imputations were only required for one subject at day 43. In this case, there was no IGA score available after Day 29, and the score on this day was used in place of a day 43 IGA score. IGA scores were re-coded, as was done to assess efficacy in the trial itself. An IGA score of 0 or 1 was considered successful treatment and IGA scores of 2-5 were counted as treatment failure. The severity of pruritus was also used as a marker of efficacy. Pruritus scores were dichotomized into absence (score = 0) or presence (score = 1, 2 or 3) of pruritus. The fisher's exact model was employed to examine the genotype effect on each efficacy variable. All statistical analyses were done using SAS version 8.2 software.

[0039] To correct for multiple testing, the Bonferroni correction method was applied to all results. The equation used to correct significance scores (p values) is: Bonferroni = $P X \eta$, where P = p value for association between genotype and efficacy of treatment and η = number of polymorphic markers genotyped in the trial.

[0040] *Demographics trial participants*. As demonstrated in Table 2, the trial subjects that consented to the pharmacogenomics (PG) analysis were representative of the patient population in the trial overall in terms of gender, age, ethnicity, and response to treatment.

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Table 2

Demographics of pharmacogenomic analysis participants compared to trial subjects

	Trial[+]		Pharmacogenomics Samples[*]	
	tacrolimus	pimecrolimus	tacrolimus	pimecrolimus
Age (years)	7.8	8.1	8.7	8.5
Race				
Caucasian	31 (41%)	45 (63%)	12 (48%)	17 (77%)
Black	14 (20%)	13 (18%)	7 (28%)	4 (18%)
Oriental	4 (6%)	3 (4%)	1 (4%)	0
Other	21 (30%)	10 (14%)	5 (20%)	1 (5%)
Gender				
male	31 (44%)	31 (44%)	12 (48%)	11 (50%)
female	39 (56%)	40 (56%)	14 (52%)	11 (50%)
IGA (baseline)[$]	3: 69 2: 1	3: 70 2: 1	3: 24	3: 21 2: 1
Efficacy (IGA)[#]	27 (40%)	22 (32%)	13 (52%)	8 (36%)
Pruritus (day 1)	0: 1 (1%) 1: 13 (19%) 2: 33 (47%) 3: 23 (33%)	0: 2 (3%) 1: 14 (20%) 2: 25 (35%) 3: 30 (42%)	1: 6 (24%) 2: 11 (44%) 3: 8 (31%)	1: 4 (18%) 2: 9 (41%) 3: 9 (41%)
Pruritus (day 43)	0: 11 (16%) 1: 35 (50%) 2: 19 (27%) 3: 3 (4%)	0: 9 (13%) 1: 35 (49%) 2: 15 (21%) 3: 10 (14%)	0: 5 (20%) 1: 10 (40%) 2: 7 (28%) 3: 3 (12%)	0: 2 (10%) 1: 14 (67%) 2: 3 (14%) 3: 2 (10%)

+ There were 70 subjects in the tacrolimus arm of the trial and 71 subjects in the pimecrolimus arm of the trial

* 24 subjects that took tacrolimus and 22 patients that took pimecrolimus were analyzed in the pharmacogenomic analysis.

$ IGA scores were taken at screening.

Number and (percent) of patients that experienced efficacy, defined as an IGA score of 0 or 1 recorded on day 43 of treatment (end of blinded portion of the trial).

[0041] *Association between (–1031) TNF and macrolactam efficacy.* As stated above, the primary efficacy variable used in statistical analysis of each genetic marker was a re-coded IGA score. A total of 31 loci in 22 genes were genotyped. Of the 31 loci that were genotyped, 5 were not polymorphic in the pharmacogenomic analysis population and were dropped from the analysis. See, Table 1, above. Thus, the penalty for multiple testing in this analysis is 26.

[0042] When the trial was analyzed as a whole (both arms combined), 1 of the 26 SNPs analyzed showed a statistically significant association with efficacy. Tumor necrosis factor alpha, *TNF*, is located in the gene-rich MHC cluster on 6p21.3 (*see*, FIG. 1), and many of the genes in this region play important roles in inflammatory processes. The promoter of *TNF* is highly

polymorphic. A SNP located –1031 bp from the transcription start site of the gene associated with efficacy (p=0.04, see Table 3, below). These data demonstrate that patients that harbor a C allele (either CC homozygotes or CT heterozygotes) at (-1031) *TNF* were less likely to respond to treatment (4/17; 24% success rate) than were TT patients (17/29; 59% success rate).

Table 3
Association between (–1031) *TNF* and macrolactam efficacy

	(-1031) *TNF* Genotype			
Response to treatment	CC	CT	TT	Total
No Success (IGA $\geq$ 2)	2 (*67%*)	11 (*79%*)	12 (*41%*)	25
Success (IGA = 0 or 1)	1 (*33%*)	3 (*21%*)	17 (*59%*)	21
Total	3	14	29	46

[0043] Table 3 demonstrates the number of subjects with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. P value for the association is 0.04 (Fisher's Exact).

[0044] We next examined the association between (–1031) *TNF* and efficacy for tacrolimus and pimecrolimus treatment independently. As seen in Table 4, those subjects who responded to pimecrolimus can be segregated from non-responders based on (-1031) *TNF*. Only subjects with a TT genotype at (-1031) *TNF* responded to pimecrolimus treatment. None of the ten subjects with a C allele (CC or CT) responded to pimecrolimus treatment. While response rate among CC and CT patients was 0%, 67% of the TTs experienced remission of their atopic dermatitis after taking pimecrolimus.

Table 4
Association between (–1031) *TNF* and pimecrolimus efficacy

	(-1031) *TNF* Genotype			
Response to pimecrolimus	CC	CT	TT	Total
No success (IGA $\geq$ 2)	1 (*100%*)	9 (*100%*)	4 (*33%*)	14
Success (IGA = 0 or 1)	0 (*0%*)	0 (*100%*)	8 (*67%*)	8
Total	1	9	12	22

[0045] Table 4 demonstrates the number of pimecrolimus treated subjects with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the

number of individuals with the given genotype that fit the response category, followed by the *percent*. P value for the association is 0.003 (Fisher's Exact).

[0046] However, genotype at this locus does not appear to influence tacrolimus efficacy. See, Table 5, below. Indeed, of the subjects that used tacrolimus, responders appear to be just as likely to be TT as CT or CC. Thus, the association seen between efficacy in the trial as a whole and (-1031) *TNF* holds only for pimecrolimus and not macrolactams in general.

Table 5
Association between (-1031) *TNF* and tacrolimus efficacy

	(-1031) *TNF* Genotype			
Response to tacrolimus	CC	CT	TT	Total
No success (IGA ≥ 2)	1 (*50%*)	2 (*40%*)	8 (*47%*)	11
Success (IGA = 0 or 1)	1 (*50%*)	3 (*60%*)	9 (*53%*)	13
Total	2	5	17	24

[0047] Table 5 demonstrates the number of tacrolimus treated subjects with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. There was no association found between (-1031) *TNF* genotype response to tacrolimus; the P value for the association is 1.0 (Fisher's Exact).

[0048] *Association between ASN60THR LTA and macrolactam efficacy*. When both arms of the trial were analyzed together, an association with efficacy was only found with (-1031) *TNF*. Interestingly, the data for a second polymorphism suggested a trend toward significance. A C→A polymorphism in exon 3 of *LTA*, which results in the production of threonine instead of asparagine at the 60th amino acid of the protein (ASN60THR), was associated with efficacy in the clinical trial (p=0.07, see Table 6, below). *LTA* is tandemly located with *TNF* on 6p21.3, and numerous reports suggest that markers in *TNF* and *LTA* are in strong linkage disequilibrium. Bouma G *et al., Scand J Immunol* 43: 456-63 (1996); Noguchi E *et al., Am J Respir Crit Care Med* 166: 43-6 (2002); .Moffatt M & Cookson W. *Hum Molec Genet* 6: 551-4 (1997); Messer G *et al., J Exp Med* 173: 209-19 (1991). Genotypes at this locus were determined in the pharmacogenomic analysis participants using an Invader Assay (TWT#229383) and confirmed by restriction digest. The data demonstrate that patients harboring an A, the wild-type allele, at

this locus were more likely to respond to treatment; the response rate among subjects with an A allele (AA or AC) was 57% (17/30), while the only 25% (4/16) of those subjects with a CC genotype experienced efficacy in the trial.

Table 6
Association between ASN60THR LTA and macrolactam efficacy (both arms combined)

	ASN60THR *LTA* Genotype			
Response to Treatment	AA	AC	CC	Total
No Success (IGA ≥ 2)	3 (*30%*)	10 (*50%*)	12 (*75%*)	25
Success (IGA = 0 or 1)	7 (*70%*)	10 (*50%*)	4 (*25%*)	21
Total	10	20	16	46

[0049] Table 6 demonstrates the number of subjects in the clinical trial with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. While a significant association was not found (P value = 0.07, Fisher's Exact), the P value approaches significance and is suggestive of a possible association.

[0050] We next examined the association between ASN60THR *LTA* and each arm of the clinical trial independently. As anticipated, a significant association was found for pimecrolimus (P=0.02, see Table 7, below), and a dosage effect was seen with the polymorphism. Patients with a CC genotype at this locus responded poorly to pimecrolimus (1/8 or 11% were successfully treated). However, patients with an AC genotype responded better (4/10 or 40% response rate), and all patients with an AA genotype responded to the treatment (3/3).

Table 7
Association between ASN60THR LTA and pimecrolimus efficacy

	ASN60THR *LTA* Genotype			
Response to pimecrolimus	AA	AC	CC	Total
No success (IGA ≥ 2)	0 (*0%*)	6 (*60%*	8 (*89%*)	13
Success (IGA = 0 or 1)	3 (*100%*)	4 (*40%*	1 (*11%*)	7
Total	3	10	9	22

[0051] Table 7 demonstrates the number of pimecrolimus treated subjects with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the

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number of individuals with the given genotype that fit the response category, followed by the *percent*. The P value for the association is 0.02 (Fisher's Exact).

[0052] A similar relationship was not found between ASN60THR *LTA* and tacrolimus efficacy. As seen in Table 8, the presence of the A allele at ASN60THR *LTA* had no influence on response to tacrolimus. As was found with (-1031) *TNF*, the association between ASN60THR *LTA* and efficacy in the clinical trial is driven by the pimecrolimus arm of the trial.

Table 8
Association between ASN60THR *LTA* and tacrolimus efficacy

Response to tacrolimus	ASN60THR *LTA* Genotype AA	AC	CC	Total
No success (IGA ≥ 2)	3 (*43%*)	4 (*40%*)	4 (*57%*)	11
Success (IGA = 0 or 1)	4 (*57%*)	6 (*60%*)	3 (*42%*)	13
Total	7	10	7	24

[0053] Table 8 demonstrates the number of tacrolimus treated subjects with each genotype that did or did not respond to treatment (referred to as "success" or "no success", respectively), as determined by IGA scores on day 43 of treatment. Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. There was no association found between ASN60THR *LTA* genotype and response to tacrolimus; the P value for the association is 1.0 (Fisher's Exact).

[0054] *Association between polymorphic markers and relief from pruritus.* A secondary efficacy variable used in the trial was pruritus. An analysis was done to see if ASN60THR *LTA* and (-1031) *TNF* associated with this efficacy variable (assessed on day 43). Results of the analysis for ASN60THR *LTA* and (-1031) *TNF* are shown in Tables 9 and 10, respectively.

[0055] Few subjects (7/46) in the pharmacogenomics group experienced relief from itching. Findings for the primary efficacy variable (IGA) suggested that the presence of a C allele at ASN60THR *LTA* decreased the likelihood of response to treatment. While a statistically significant association between ASN60THR *LTA* and pruritus was not found, no subject that was homozygous CC at this locus experienced relief from itching as a result of treatment in this trial. For (-1031) *TNF*; while analysis of the primary efficacy variable IGA suggested that TT subjects were far more likely to respond to treatment, the same cannot be said when pruritus scores are analyzed for the trial as a whole. The two arms of the trial could not be separated for analysis of pruritus because the number of responders is too small. Only two pimecrolimus subjects in the

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pharmacogenomic analysis samples experienced relief from itching. However, both of the pimecrolimus subjects that experienced efficacy with respect to pruritus were TT at (-1031) *TNF*.

Table 9
Association between ASN60THR *LTA* and pruritus (both arms combined).

Response to treatment	ASN60THR *LTA* Genotype AA	AC	CC	Total
No Success (Pru=1,2,3)	7 (*70%*)	16 (*80%*)	16 (*100%*)	39
Success (Pru=0)	3 (*30%*)	4 (*20%*)	0 (*0%*)	7
Total	10	16	16	46

[0056] Table 9 demonstrates the number of subjects with each genotype at the ASN60THR *LTA* locus studied that did or did not respond to treatment, as determined by pruritus scores on day 43 of treatment. Treatment was considered successful if the patient reported no pruritus (score = 0). Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. A significant association was not found between ASN60THR *LTA* genotype and relief from pruritus (P=0.06, Fisher's Exact), however the P value for the association approached significance.

Table 10
Association between (-1031) *TNF* and pruritus following treatment (both arms combined)

Response to treatment	(-1031) *TNF* Genotype CC	CT	TT	Total
No success (Pru=1,2,3)	3 (*100%*)	12 (*86%*)	24 (*83%*)	39
Success (Pru = 0)	0 (*0%*)	2 (*14%*)	5 (*17%*)	7
Total	3	14	29	46

[0057] Table 10 demonstrates the number of subjects with each genotype at (-1031) *TNF* that did or did not respond to treatment, as determined by pruritus scores on day 43 of treatment. Treatment was considered successful if the patient reported no pruritus (score = 0). Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. No association was found (P=1.0, Fisher's Exact).

[0058] *Genotypes at (-1031) TNF and ASN60THR LTA*. Genotypes of anonymized patients at these two 6p21.3 loci were obtained. Only subjects with a TT genotype at (-1031) TNF responded to pimecrolimus therapy. The observation that individuals that are TT homozygotes for (-1031) TNF are either AA homozygotes, AC heterozygotes, or CC homozygotes or for

ASN60THR LTA show that (-1031) TNF and ASN60THR are not in complete linkage disequilibrium.

Table 11
Nucleotide sequence surrounding the ASN60THR LTA and (-1031) TNF polymorphisms

Gene	Allele 1	Allele 2	Surrounding Sequence
LTA	C	A	GTGAGCAGCAGGTTTGAGG[C,A]TGCTGTGGGCAA GATGCATCTTGGGGTG (SEQ ID NO: 9)
TNF	T	C	AGCAAAGGAGAAGCTGAGAAGA[T,C]GAAGGAAA AGTCAGGGTCTGGAGGGGCGGG (SEQ ID NO: 10)

[0059] *Associations between pimecrolimus and tacrolimus and other markers typed.* All of the other molecular markers typed were checked to see if they segregated responders from non-responders for each arm of the trial independently. No additional associations were found for pimecrolimus, though one association was found for tacrolimus. A G→A polymorphism in the coding region of *CCR2* (TWT #47987), which results in a change in a valine to isoleucine (VAL64ILE), associated with response to treatment with tacrolimus (p=0.04, see Table 11, below). The P value for the association between pimecrolimus efficacy and VAL64ILE *CCR2* was 1.0 (Fisher's Exact).

[0060] *CCR2* encodes chemokine (C-C) motif receptor 2, a receptor for monocyte chemoattractant protein-1 and a chemokine that mediates monocyte infiltration in inflammatory diseases. *CCR2* encodes for both known isoforms of the receptor for monocyte chemoattractant protein-1 (MCP-1, also known as SCYA2), a chemokine which specifically mediates monocyte chemotaxis. MCP-1 has been shown to be involved with inflammatory diseases like rheumatoid arthritis and atherosclerosis. Boring L *et al., Nature* 394:894-7 (1998). The VAL64ILE polymorphism occurs in the first transmembrane region of CCR2 and has been studied in the context of HIV-1 infection and AIDS. Mummidi S *et al., Nature Med* 4: 786-93 (1998). The association found was p=0.04.

[0061] These data demonstrate that an A allele at VAL64ILE *CCR2* were more likely to respond to tacrolimus treatment; 8/10 or 80% of AG subjects experienced efficacy, as compared to 4/13 or 31% of GG subjects. (There were no AA subjects in the population). This association is weaker than the associations seen with pimecrolimus efficacy, particularly for (-1031) *TNF* and pimecrolimus efficacy (p=0.003).

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Table 12
Association between VAL63ILE *CCR2* (TWT# 47987) and tacrolimus efficacy

Response to tacrolimus	VAL63ILE *CCR2* Genotype AA	AG	GG	Total
No success (IGA $\geq$ 2)	0	2 (*20%*)	9 (*69%*)	11
Success (IGA = 0 or 1)	0	8 (*80%*)	4 (*31%*)	12
Total	0	10	13	23

[0062] Table 12 demonstrates the number of tacrolimus treated subjects with each genotype at VAL63ILE *CCR2* that did or did not respond to treatment (determined by IGA scores on day 43 of treatment). Each square contains the number of individuals with the given genotype that fit the response category, followed by the *percent*. P value for the association was 0.04 (Fisher's Exact).

[0063] *LTB* (TWT # 128095) is also located in the *TNF* gene cluster on 6p21.3. A polymorphism in *LTB* was also typed in this analysis. As shown below, however, there is no association between TWT#128095 and efficacy in the clinical trial. No association was found when pimecrolimus and tacrolimus subjects were analyzed independently, either.

Table 13
Relationship between efficacy in trial (both arms combined) and *LTB* (TWT#128095); data demonstrate no association (p=1.0, Fisher's Exact)

Response to treatment	*LTB* (TWT#128095) AC	CC	Total
No success (IGA $\geq$ 2)	3 (*50%*)	20 (*54%*)	23
Success (IGA = 0 or 1)	3 (*50%*)	17 (*46%*)	20
Total	16	26	43

Table 14
Relationship between efficacy in among pimecrolimus-treated subjects and *LTB* (TWT#128095); data demonstrate no association (p=1.0, Fisher's Exact)

Response to pimecrolimus	*LTB* (TWT#128095) AC	CC	Total
No success (IGA $\geq$ 2)	0 (*50%*)	12 (*63%*)	12
Success (IGA = 0 or 1)	1 (*100%*)	7 (*37%*)	8
Total	1	19	20

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Table 15
Relationship between efficacy in among tacrolimus-treated subjects and *LTB* (TWT#128095); data demonstrate no association (p=1.0, Fisher's Exact)

Response to tacrolimus	*LTB* (TWT#128095) AC	CC	Total
No success (IGA ≥ 2)	3 (*60%*)	8 (*44%*)	11
Success (IGA = 0 or 1)	2 (*40%*)	10 (*56%*)	12
Total	5	18	23

[0064] These data help to localize the genetic region that is responsible for response to pimecrolimus; the biologically relevant polymorphism does not appear to be localized proximal to *TNF*.

[0065] In summary, either *LTA* or *TNF* plays an important role in determining efficacy of pimecrolimus in the treatment of pediatric atopic dermatitis. By contrast, response to tacrolimus appears to be influenced by other biological pathways. Thus, different biological mechanisms influence response to pimecrolimus and tacrolimus. While pimecrolimus and tacrolimus have substantial structural similarities and both target macrophilin-12 with the ultimate effect of inhibiting calcineurin, the two compounds are known to have distinct properties. Nghiem P *et al., J Am Acad Dermatol* 46: 228-241 (2002) and references cited therein.

[0066] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In addition, all GenBank accession numbers, Unigene Cluster numbers and protein accession numbers cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each such number was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

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[0067] The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The present invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

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CLAIMS

We claim:

1. A method for treating a condition in a subject, wherein the condition is selected from the group consisting of atopic dermatitis, psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or other condition for which pimecrolimus or tacrolimus is indicated, comprising the steps of:
   (a) obtaining the genotype of a subject at a genetic locus from the TNF gene cluster indicative of efficacy of a selected macrolactam formulation in treating the condition;
   (b) administering either the selected macrolactam formulation or an alternative treatment for the condition to the subject.

2. The method of claim 1, wherein the selected macrolactam formulation comprises pimecrolimus.

3. The method of claim 1, wherein the selected macrolactam formulation comprises tacrolimus.

4. The method of claim 1, wherein the genetic locus is the human *TNF* gene or a gene in a vertebrate species homologous to the human *TNF* gene.

5. The method of claim 1, wherein the genetic locus is the (-1031) *TNF* locus of the human *TNF* gene or a corresponding locus in a vertebrate species homologous to the human *TNF* gene.

6. The method of claim 1, wherein the selected macrolactam formulation comprises pimecrolimus, when the (-1031) *TNF* genetic locus has a TT genotype.

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7. The method of claim 1, wherein the alternative treatment for the condition is selected from the group consisting of a high dose of pimecrolimus, pimecrolimus and an alternative immunosuppressant, and an alternative immunosuppressant, when the (-1031) *TNF* locus has a CC or CT genotype.

8. The method of claim 7, wherein the alternative immunosuppressant is selected from the group consisting of hydrocortisone, cyclosporine, tacrolimus and sirolimus.

9. The method of claim 1, wherein the genetic locus is the human *LTA* gene or a gene in a vertebrate species homologous to the human *LTA* gene.

10. The method of claim 1, wherein the genetic locus is the ASN60THR *LTA* locus of the human *LTA* gene or a corresponding locus in a vertebrate species homologous to the human *LTA* gene.

11. The method of claim 1, wherein the selected macrolactam formulation comprises pimecrolimus, when the ASN60THR *LTA* genetic locus has an AA or AC genotype.

12. The method of claim 1, wherein the alternative treatment for the condition is selected from the group consisting of a high dose of pimecrolimus, pimecrolimus and an alternative immunosuppressant, and an alternative immunosuppressant, when the ASN60THR *LTA* locus has a CC genotype.

13. The method of claim 12, wherein the alternative immunosuppressant is selected from the group consisting of hydrocortisone, cyclosporine, tacrolimus and sirolimus.

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14. A method for treating a condition in a subject, wherein the condition is selected from the group consisting of atopic dermatitis, psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or other condition for which pimecrolimus or tacrolimus is indicated, comprising the steps of:

(a) obtaining the genotype of a subject at a genetic locus indicative of efficacy of a selected macrolactam formulation in treating the condition, where the genetic locus is the human *CCR2* gene or a gene in a vertebrate species homologous to the human *CCR2* gene;

(b) administering either the selected macrolactam formulation or an alternative treatment for the condition to the subject.

15. The method of claim 14, wherein the genetic locus is the VAL64ILE *CCR2* locus of the human *CCR2* gene or a corresponding locus in a vertebrate species homologous to the human *CCR2* gene.

16. The method of claim 14, wherein the selected macrolactam formulation comprises tacrolimus, when the VAL64ILE *CCR2* genetic locus has a GG genotype.

17. The method of claim 14, wherein the alternative treatment for the condition is selected from the group consisting of a high dose of tacrolimus, tacrolimus and an alternative immunosuppressant, and an alternative immunosuppressant, when the VAL64ILE *CCR2* locus has an AG genotype.

18. The method of claim 17, wherein the alternative immunosuppressant is selected from the group consisting of hydrocortisone, cyclosporine, pimecrolimus and sirolimus.

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19. A method for treating a condition in a subject, wherein the condition is selected from the group consisting of atopic dermatitis, psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or other condition for which pimecrolimus is indicated, comprising the steps of:

(a) obtaining a measurement of the level of TNF-α mRNA in a sample from a subject, where the sample is taken from non-inflamed tissue; and

(b) either:

(i) administering a pimecrolimus formulation to the subject when the level of TNF-α mRNA in the sample is low or normal; or

(ii) administering either (A) a pimecrolimus formulation in combination with another immunosuppressant or (B) another immunosuppressant to the subject when the level of TNF-α mRNA in the sample is low or normal.

20. A method for treating a condition in a subject, wherein the condition is selected from the group consisting of atopic dermatitis, psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or other condition for which pimecrolimus is indicated, comprising the steps of:

(a) obtaining a measurement of the level of TNF-α protein in a sample from a subject, where the sample is taken from non-inflamed tissue; and

(b) either:

(i) administering a pimecrolimus formulation to the subject when the level of TNF-α protein in the sample is low or normal; or

(ii) administering either (A) a pimecrolimus formulation in combination with another immunosuppressant or (B) another immunosuppressant to the subject when the level of TNF-α protein in the sample is low or normal.

21. A method for choosing subjects for inclusion in a clinical trial for determining the efficacy of a pimecrolimus formulation, comprising the steps of:

(a) wherein the (-1031) TNF polymorphism is interrogated.

(b) then:

(i) including in the trial if they are TT at this locus;

(ii) excluded if they are CC or CT at this locus; or

(iii) both (i) and (ii).

22. A kit for use in determining a treatment strategy for a condition, wherein the condition is selected from the group consisting of atopic dermatitis, psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis or other condition for which pimecrolimus or tacrolimus is indicated, comprising:

(a) a reagent for detecting a biomarker of efficacy of treatment of the condition by a macrolactam formulation;

(b) a container for the reagent; and

(c) a written product on or in the container describing the use of the biomarker in determining a treatment strategy for the condition.

23. The kit of claim 22, wherein the biomarker is a genetic polymorphism in a gene selected from the group consisting of *TNF*, *LTA* and *CCR2*.

24. The kit of claim 23, wherein the reagent for detecting the biomarker is a set of primer pairs that hybridize to a polynucleotide on either the side of the genetic polymorphism in the gene selected from the group consisting of *TNF*, *LTA* and *CCR2*and which define a nucleotide region that spans the genetic polymorphism.

25. The kit of claim 22, wherein the biomarker is the level of TNF-α mRNA in a sample from a subject to be treated.

26. The kit of claim 22, wherein the biomarker is the level of TNF-α protein in a sample from a subject to be treated.

ABSTRACT

The MHC III region of chromosome 6p21.3 harbors a DNA sequence, most likely within the *TNF* or *LTA* gene, which influences response to pimecrolimus for the treatment of atopic dermatitis. Accordingly, genetic polymorphisms in the *TNF* and *LTA* genes are useful as biomarkers of the efficacy of pimecrolimus treatment of inflammatory disease. By contrast, response to tacrolimus appears to be influenced by other biological pathways.

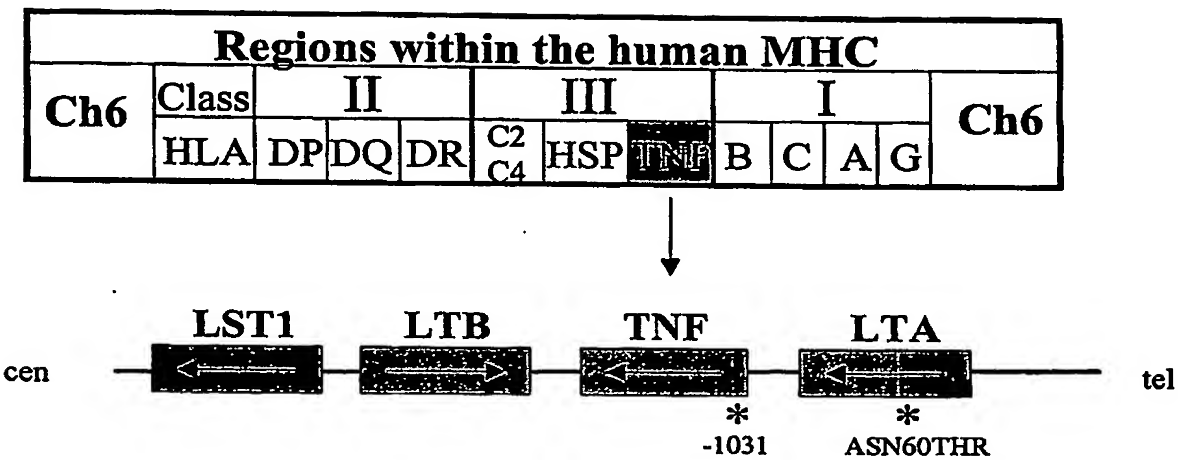
Regions within the human MHC
Ch6
Class
II
III
I
Ch6
HLA
DP
DQ
DR
C2
C4
HSP
TNF
B
C
A
G
LST1
LTB
TNF
LTA
cen
tel
*
-1031
*
ASN60THR

FIG. 1